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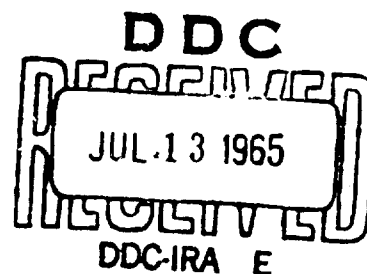
A STUDY OF PLAGUE BACTERIOPHAGES AND BACTERIAL
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A STUDY OF PLAGUE BACTERIOPHAGES
AND BACTERIAL MUTANTS RESISTANT THERE TO.
I. BIOLOGICAL PROPERTIES OF PLAGUE
BACTERIOPHAGES

[Following is the translation of an article by A. I. Volosivets in the Russian-language journal Byulleten' Eksperimental'noy Biologii i Meditsiny (Bulletin of Experimental Biology and Medicine), No 9, 1963, pages 81-84.]

From the All-Union Scientific Research Scientific Research "Mikrob" Institute (Director: Professor N. I. Nikolayev), Saratov

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Numerous investigations have established that bacterial cultures are nonuniform in their capacity to adsorb phages, and also in their sensitivity thereto. In light of modern data, phage-resistance is acquired in the lysogenization of bacteria or as a result of their mutation. Characteristics determining the emergence of resistance upon lysogenization are clear enough, since they are associated with the penetration into the cell of DNA of the temperate phage. However, with respect to phage-resistance arising as the result of mutations, two suggestions are known. According to one of these, phage-resistant mutants are the result of spontaneous mutations of bacteria independently of their contact with phage, the role of which amounts only to selection of resistant variants. According to the other hypothesis, resistant mutants appear by virtue of hereditary changes of cells resulting from their contact with the phage (adaptation). The

mutational origin of phage-resistant variants has been shown for intestinal bacillus (12, 13, 14). With respect, however, to other species of bacteria, especially pathogenic, such a suggestion has raised objections. Therefore, study of the origin of phage-resistance among various species of bacteria together with studies of the properties of phages and phage-resistant variants is one of the approaches to finding out the general regularities governing the character of bacterial phagia.

We present in this report results of a study of biological properties of plague phages (EB, 1-17, d'Erelle), which were used to obtain phage-resistant variants of plague bacteria and to elucidate their origin. The properties studied include the range of phage action, phage specificity, adsorptive capability, duration of intracellular development, and average yield per one bacterial cell.

Experimental Methods

Sensitivity to the phages EB, 1-17, and d'Erelle was verified on 100 strains of plague bacillus of differing virulence in the R-, OR-, and OS-forms (4) by applying a drop of undiluted phage on the gazon [?] of an 18-hour culture and its subsequent distribution in droplet trails, and also by the method of agar layers using diluted phages (also 100 strains). Other properties (adsorptive capacity, duration of intracellular development, and average yield per cell) were studied following methods described by Adams (8,10). In experiments aimed at revealing the adsorptive capacity of the latent period and the yield use was made of casein broth.

Experimental Results

In studying strains of plague causative agent isolated in mountain and plain foci (Central Asia), we did not succeed in finding among these any phage-resistant bacterial variants (Table 1).

As we can see from Table 1, all strains, independently of where they were isolated from and their virulence, proved sensitive to available phages.

In the literature, there is described the capability of plague phages to lyse causative agents of pseudotuberculosis, representatives of salmonellosis, and hemorrhagic septicemia (1, 2,5, 7). To reveal the specificity of the phages studied, EB, 1-17, and d'Erelle tested on avirulent indicator culture (EB) 211 strains of plague bacillus causative agent were used in the experiment, 40 strains of pseudotuberculosis, and 202 of various infections (paracholeric Vibro, salmonellosis dysentery bacilli, brucellosis, pasteurella, listerella, spore, capsular, pigment-forming cocci, etc.). As experimental results indicated, in addition to plague causative agents certain strains of pseudotuberculosis also proved sensitive to phages. The percentage of lysed representatives of pseudotuberculosis causative numbered ten for the phages

EB and d'Erell and 12 for the phages 1-17. Among the lyzed strains of pseudotuberculosis causative there were included R- and OR- forms, among the resistant -- R-, OR-, and S- forms. Representatives of other species of microroganisms were totally unlyzed. Such specificity is evidently the result of the raising of phages on a single strain (EB).

TABLE 1
Range of Action of Plague Phages (number of cultures lyzed
by the phages)

a) Очаг	d) Характери- стики культур по вирулент- ности	h) Наименование фазов и степень их разведения					
		EB		d'Эрелля		1-17	
		10 ⁴	10 ⁷	10 ⁴	10 ⁷	10 ⁴	10 ⁷
b) Равни- на	e) Авирулент- ные	15		15		2	13
	f) Вирулент- ные ¹	50		50		7	43
c) Гор- ный	g) Авирулент- ные	14		14		1	13
	Вирулент- ные	21		21		2	19
	EB-авиру- лентный	1(70) ²		1(110) ²		1(20) ²	

¹ Regarded as virulent strains of plague causative are those which induced death of guinea pigs during 4-20 days.

² In parentheses is given the number of negative colonies on the EB strain.

LEGEND: a) focus; b) plain; c) mountains; d) characteristics of culture as to virulence; e) avirulent; f) virulent¹; g) EB-avirulent; h) kind of phage and extent of dilution; i) d'Erelle.

In the experiments studying the absorptive capacity of phages, it was found that most phage corpuscles are adsorbed during five minutes in the case of the phages EB and 1-17, and in 8 minutes -- for the phage d'Erelle. However, in certain experiments, the main bulk of the phage particles was adsorbed by the eighth minute for the phages EB and 1-17, and by the tenth minute -- for the phage d'Erelle. In the subsequent period, the number of phage particles remained constant or varied slightly toward the greater or lesser side.

TABLE 2

Adsorptive Capacity of Plague Phages

a) Фаз	b) Адсорбция фазов (в %) после контакта в течение					c) Константа скорости адсорбции фага (в млн/мин)
	1 минуты	3 минут	4 минут	5 минут	8 минут	
EB	25.9	45.9	54.2	66.7	66.7	$4.4 \cdot 10^8$
1-17	47.1	50.6	64.2	73	73	$5.2 \cdot 10^8$
д'Эрелли	31.7	38.7	54.6	59.2	72.8	$3.2 \cdot 10^8$

LEGEND: a) phage; b) adsorption of phages (in per cent) after contact for time listed; c) phage adsorption rate constant (in millions/minute); d) minute; e) minutes; f) d'Erelle.

From Table 2, it is clear that the adsorption dynamics and rate constant for the phages studied are dissimilar. The highest adsorption rate constant was noted for the phage 1-17 (titer $11 \cdot 10^8$), the next highest -- for the phage EB (titer $= 7 \cdot 10^8$), and the lowest -- for the phage d'Erelle (titer $2 \cdot 10^8$).

Thus, the adsorptive capacity of plague, as well as many other, phages, lies in correlation with lytic activity.

The next stage of the study was exploration of the inter-relationship between lytic activity of phages and the duration of their intracellular development, and also their productivity (Table 3).

In view of the fact that the sizes of the negative plague phage colonies significantly exceed the values for other phages and rapidly merge together, their count was hampered within the limits of the first two-three hundred. For this reason, the choice of the end of the latent period was the time when after six hours of incubation at 28° individual negative colonies could still be counted, but during the following interval, their merging was observed. In accordance with the adopted definition, the end of the latent period for the bacterial phages EB and 1-17 set in by the 23rd minute, and by the 25th minute for the phage d'Erelle. As was true for the adsorptive capacity, the period of intracellular phage development correlated with the lytic activity.

In addition to finding out the average yield in the experiment of a single developmental cycle, this variable was also studied in individually isolated bacteria (10). It was shown that the average yield for the EB bacterial phage was 110 particles per cell, for the phage 1-17 -- 72 phage particles, and for d'Erelle -- 54. Recapitulating the results, we can note that the mean yield of the

phages EB and d'Erelle when verified by two methods remained more constant (EB: 100-106-110; d'Erelle: 51-54-56), while the yield of bacterial phage 1-17 ranged within the limits 72-95 phage particles per bacterium.

TABLE 3

Latent Period and Yield of Plague Phages

a) Phage	b) Скрытый период развития (в минутах)	c) Среднее количество фage-частиц на одну клетку
EB	23	100
1-17	23	95
d'Erelle	25	56

LEGEND: a) phage; b) latent developmental period (in minutes); c) mean number of phage particles per single cell; d) d'Erelle.

It is known that one of the most reliable taxonomic features for classifying bacterial phages is their antigenic properties (9), which are determined in experiments in which they are neutralized by their antiphage sera. The neutralization reaction is characterized by the variable K -- the neutralization rate constant. The capacity of the antiphage sera to neutralize phages has been studied in serial dilutions of 1:10, 1:20, 1:40, 1:80, and 1:160. Experimental results evidence that the antiphagal sera obtained exhibited low activity and neutralized 90-99 % of phage particles in a dilution of 1:10 in the course of five and ten minutes. Data on phage neutralization by homologous and heterologous sera are presented in Table 4.

As the experimental results show, antiphagal sera of one phage neutralize all other phages at almost the same intensity, which points to a kinship of the plague phages studied and agrees with the data of other authors (3, 6). This allows us to combine them into a single serological group. The rate at which phage particles are neutralized by homologous sera is higher than the rate for heterologous. The variable K for the phages studied ranged within the limits 1.9-6.1. No relationship was established in the experiments between lytic activity and antigenic properties of phages, which confirms the existing point of view on the separation between the receptor and antigenic structures of phage particles.

Thus, the bacterial phages studied, EB, 1-17, and d'Erelle, exhibit a broad range of action with respect to plague bacteria of different origins, but lyse also pseudotuberculosis causative agents (10-12 %). Adsorption of plague phages occurs during the course of 5-8 minutes (EB and 1-17) and 8-10 minutes (d'Erelle). The latent developmental period of the phages EB and 1-17 is 23 minutes, and

TABLE 4

Serological Properties of Plague Phages

a) Фар	b) Время контакта (в минуты)	в) Идентификация сыворотки (в 1:10 разведении) по реакции агглютинации					
		г) Анти-EB	д) К	е) Анти-1-17	ж) К	з) Анти-д'Эрелле	и) К
EB	5	95.4	6.1				
	10	98.5	4.1	97.5	3.6	87.5	2.0
1-17	5			86	3.9	86	
	10	95.2	3.0	98	3.9	92	
д'Эрелле	5						4.8
	10	98.4	4.1	96.4	3.3	93	2.0

LEGEND: a) phage; b) duration of contact (in minutes); c) phage neutralization (in per cent) by antiphagal sera in a 1:10 dilution; d) d'Erelle; e) antisera to EB; f) antisera to 1-17; g) antisera to d'Erelle.

25 minutes for the d'Erelle bacterial phage. The latter two characteristics are related with the lytic activity of phages. These phages constitute a single serological group.

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